Polymicrobial wound infections: Pathophysiology and current therapeutic approaches

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ABSTRACT

Acute and chronic wounds represent a very common health problem in the entire world. The dermal wounds are colonized by aerobic and anaerobic bacterial and fungal strains, most of them belonging to the resident microbiota of the surrounding skin, oral cavity and gut, or from the external environment, forming polymicrobial communities called biofilms, which are prevalent especially in chronic wounds. A better understanding of the precise mechanisms by which microbial biofilms delay repair processes together with optimizing methods for biofilm detection and prevention may enhance opportunities for chronic wounds healing. The purpose of this minireview is to assess the role of polymicrobial biofilms in the occurrence and evolution of wound infections, as well as the current and future preventive and therapeutic strategies used for the management of polymicrobial wound infections.

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1. Introduction

Acute and chronic wounds affect millions of people in the entire world (Demidova-Rice et al., 2012). The acute wound repair process has four phases partially superposed in time and space, *i.e.* (i) coagulation (during this phase, the platelets adhere to the damaged blood vessels and initiate the blood-clotting cascade with hemostatic and protective roles; the platelets are also releasing chemotactic factors for mononuclear and polymorphonuclear phagocytes) (Singer and Clark, 1999; Weyrich and Zimmerman, 2004); (ii) inflammation (this process is mediated by the arrived leukocytes, which are releasing reactive oxygen species with microbicidal activity and proteases that clear the wound of foreign bodies, devitalized tissues and microbial cells; the inflammation is resolved in few days, being accompanied by the apoptosis of inflammatory cells) (Gilroy et al., 2004; Eming et al., 2007); (iii) formation of granulation tissue

(this phase is mediated by the proliferation of dermal and epidermal cells and the activation of a strong angiogenic response requiring the activation of endothelial progenitor cells, and having as consequence the synthesis of the extra-cellular matrix forming granulation tissue containing mainly collagen I, irrigated by the newly formed blood vessels) (Humar et al., 2002; Liu and Velazquez, 2008), and iv) remodeling or scar formation phase (this phase is involving wound contraction mediated by differentiated fibroblasts or myofibroblasts, which acquire a smooth muscle actin-containing stress fibers phenotype, matrix remodeling by matrix metalloproteases and scar formation following apoptosis of fibroblastic cells) (Hinz, 2007; Rai et al., 2005).

Chronic wounds, divided in vascular (*e.g.* venous and arterial ulcers), diabetic and pressure ulcers, are characterized by a common sequence of processes impairing the wound healing, such as: (i) prolonged or excessive inflammatory phase (Eming et al., 2007); (ii) persistent infections, with the formation of drug-resistant microbial biofilms (Wolcott et al., 2008) and (iii) the inability of dermal and/or epidermal cells to respond to reparatory stimuli.

2. Pathophysiology of polymicrobial wound infections

The skin microbiota analysis by advanced molecular methods (pyrosequencing) indicate that a small percent of 1–2% of the skin colonizing microorganisms can be cultivated and also that there is a significantly lower diversity of bacteria found in wounds than in the intact skin, demonstrating the barrier role of the normal microbiota against colonization, proliferation and dissemination of opportunistic and pathogenic microorganisms (Martin et al., 2010; Gontcharova et al., 2010).

The loss of skin integrity favors the exposure of subcutaneous tissue to microbial colonization and proliferation, by providing appropriate moisture, temperature and nutritive conditions. The presence of foreign bodies and of devitalized tissue facilitates microbial proliferation in the absence of early prophylactic antibiotic treatment and surgical debridement (Robson, 1997).

Microorganisms that colonize the damaged tissues often form polymicrobial communities called biofilms, that may be defined as varied consortia of fungi, bacteria, and viruses that exist at a phase or density interface embedded in a self-secreted and/or host-derived, self-hydrated polymer matrix, often consisting of polysaccharides (Brogden et al., 2005), which provides an optimal environment for microbial cells survival, enabling their escape from host immune system and resistance to antibiotic treatment (James et al., 2008; Martin et al., 2010). The presence of one microorganism generates an appropriate environment for other pathogenic microorganisms, which are able to colonize the respective niche, while two or more non-pathogenic microorganisms could synergically interact to cause disease. This is possible because of the fact that the components of the normal microbiota, with a large variety and density, co-evolved for a very long period of time in a relatively small space, favoring the establishment of complex and specific physical and chemical interactions.

The ecological interactions established among the members of microbial associations could be: (i) mutualistic or synergistic, facilitating the adherence to the epithelial surfaces and the efficient utilization of nutrients and metabolic by-products or (ii) competitive/antagonistic (Brian et al., 2012) implicated in many processes, such as: contact-dependent attachment, intercellular communication via quorum-sensing cross-talk, colonization enhancement, augmented/changed virulence phenotypes, immunomodulation (Peleg et al., 2010).

Similarly to the virulence concept, that can no longer be associated with a single virulence factor for certain pathogens, some infectious diseases, such as the biofilm-associated ones, can no longer be attributed to a monospecific etiology (Casadevall and Pirofski, 2001). The microbial density, species, associations, as well the host immune response are all predictive factors for the wound healing and infection.

Microbial biofilms are prevalent especially in chronic wounds, such as diabetic foot, pressure, and venous leg ulcers, being often constituted by diverse polymicrobial communities, including aerobic as well as not cultivable, strictly anaerobic bacteria (James et al., 2008; Bowler and Davies, 1999; Bowler, 1998).

The dermal wounds are colonized by aerobic and anaerobic bacterial and fungal strains, most of them belonging to resident microbiota of the surrounding skin, oral cavity and gut, or from the external environment (Bowler et al., 2001). It is considered that aerobic or facultative pathogens such as Staphylococcus aureus, coagulase-negative staphylococci, Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp., Enterobacter spp., Enterococcus spp. and beta-hemolytic streptococci, as well as Candida spp. are the primary causes of delayed healing and infection in both acute and chronic wounds, especially the surgical ones (Duerden, 1994; Mangram et al., 1999). The anaerobic Bacteroides fragilis, Clostridium perfringens, Porphyromonas spp., Peptostreptococcus spp. and Prevotella spp. are the most cited species involved in soft tissues and bite wound infections (Brook, 1996, 1998; Brook and Randolph, 1981; Brook and Frazier, 1990, 1997, 1998a,b; Brook and Finegold, 1981; Bariar et al., 1997).

In diabetes mellitus, the high glucose blood levels lead to osmotic imbalance, dehydration of body tissues, and, if not treated properly, to organ damage (Alberti and Zimmet, 1998), development of peripheral neuropathy and poor blood circulation, especially in limbs, predisposing to an increased risk of infection, with chronic evolution generating ulcerating polymicrobial biofilm-mediated wounds. Due to the inability of diabetic subjects to feel cuts and irritations on visually obscured areas of the feet, these infections often pass unnoticed and progress to more serious illnesses, hard to treat and potentially leading to limb amputation (Wu et al., 2007; Boulton, 2010).

The most abundant aerobic isolates recovered from this kind of infections were Corynebacterium spp., Enterococcus spp., E. coli, Staphylococcus epidermidis, and S. aureus; among the most commonly isolated anaerobic bacteria were Fusobacterium spp., Porphyromonas spp., Prevotella spp., Bacteroides spp., and Clostridium spp., often associated in polymicrobial communities with a large diversity (Gardner and Frantz, 2008).

The development of polymicrobial aerobic–anaerobic populations is facilitated by the low oxygen tension (hypoxia or anoxia) and the reduced redox potential of the wound environment (Gerding, 1995).

The wound evolution in diabetic patients is aggravated by the defects in wound healing (i.e. the inability of keratinocytes to migrate and differentiate properly, inappropriate levels of angiogenic and growth factors, epidermal barrier function, fibroblast migration, and macrophage function (Falanga, 2005; Galkowska et al., 2006; Maruyama, 2007). It was demonstrated that the presence of biofilm-forming S. aureus strains specifically inhibit wound-healing mechanisms and exacerbate disease (Bowling et al., 2009).

Wound infection is the result of virulence factors expression, surpassing the host natural immune system, followed by microbial dissemination and tissue invasion, leading to the occurrence of a local purulent discharge, pain or erythema (Peel, 1992). Live bacteria and their toxins induce excessive inflammatory responses and tissue damage that can lead to abscesses, cellulitis, osteomyelitis or limb loss (Ovington, 2003). The recruited inflammatory cells, as well as bacteria, produce different proteases, which could degrade the ECM and also, growth factors.

A better understanding of the precise mechanisms by which bacterial biofilms delay repair processes together with optimizing methods for biofilm detection and prevention may enhance opportunities for chronic wounds healing. Taking into account that the strictly anaerobic or fastidious microorganisms cannot be recovered by culture methods, combination of molecular and culturing methods is required in order to obtain a more complete characterization of the microbial diversity of chronic wounds, and a deeper understanding of the role of different microbial species in chronic wound pathology and healing (Frank et al., 2009; Gontcharova et al., 2010).

Many studies have been stated that a microbial load of $\geq 10^6$ organisms per quantitative swab sample taken from open burn wounds when bacterial cells were observed in a Gram-stained smear prepared from the same sample or $>5 \times 10^4$–10^5 CFU/g of tissue is predictive for the occurrence of a wound infection (Breidenbach and Trager, 1995; Levine et al., 1976; Raahave et al., 1986).

On the other side, the presence of more bacterial species is associated with a higher probability of wound infection, due to the synergic interactions between species, which could favor, for example, the occurrence of a hypoxic environment appropriate for

the development of anaerobic species, as a consequence of oxygen consumption by aerobic species; ability of some species to provide metabolites required for the growth of fastidious microorganisms or to impair the immune defense system (Bowler and Davies, 1999; Kingston and Seal, 1990; Trengove et al., 1996).

Limited studies have specifically addressed interactions within multi species biofilms and particularly interactions between bacteria and fungi, which are often found together in different environments (Joint et al., 2002; Hogan, 2006; Shirtliff et al., 2009). Recently, the contribution of fungal pathogens to mixed species biofilms has been assessed due to the extremely recalcitrant nature of many wounds to antibacterial agents, fungi contributing to >50% of the microbial burden in the majority of the wounds (Harriott and Noverr, 2011), the most prevalent being the genus *Candida*; but also the genera *Curvularia*, *Malessezia*, *Aureobasidium*, *Cladosporium*, *Ulocladium*, *Engodontium* and *Trichophyton*. Although the area of research exploring interkingdom interactions in biofilm is still at the beginning, there is increasing awareness of their clinical implications particularly concerning the relationships that the fungal pathogen *Candida albicans* establishes with various bacterial species. *C. albicans* is the major fungal pathogen of humans causing a variety of afflictions ranging from superficial mucosal diseases to deep-seated mycoses (Seneviratne et al., 2008).

A recent study examining the structure of biofilm formed by *C. albicans* and the bacterial pathogen *S. aureus* revealed a unique architecture when *S. aureus* were associated with the hyphae of *C. albicans*. Interestingly, a number of proteins demonstrated a significant level of differential protein expression and were identified to be virulence factors in *S. aureus*, indicating a process whereby *C. albicans* may enhance *S. aureus* pathogenesis. Among the upregulated staphylococcal proteins was L-lactate dehydrogenase 1, which confers resistance to host-derived oxidative stressors (including to oxidative substances from phagocytes). Among the downregulated proteins was the global transcriptional repressor of virulence factors, CodY.

S. aureus, and to a lesser extent *E. coli* and *Klebsiella pneumoniae* promote the growth and virulence expression of vitamin K-dependent strains of *Prevotella melaninogenica*, *Prevotella loescheii* and *Porphyromonas gingivalis* (Ingham et al., 1977; Mayrand and McBride, 1980; McKee et al., 1986), by providing hemin, vitamin K, succinate. Mayrand and McBride (1980) suggested that succinate production by *K. pneumoniae* is enhanced in the presence of glucose, explaining the increased prevalence of wound infection rates in diabetic patients (Rubinstein et al., 1983). The increased levels of succinate produced by Gram-negative bacteria will subsequently impair host cell function and render the host more susceptible to infection (Rubinstein and Pierce, 1988). Succinate is also more active at acidic pH, and therefore the hypoxia and low pH associated with many chronic wounds will facilitate succinate activity and hence exacerbate impaired neutrophil function. A synergistic interaction between *S. aureus* and *S. pyogenes*, and non-sporing anaerobes has been recognized in various types of non-clostridial cellulitis and necrotizing fasciitis (Kingston and Seal, 1990).

In diabetes foot ulcer, the simultaneous infection with many species led to the highest rate of mortality (Mastropaolo, 2005). A synergic pathogenicity was observed for *E. coli* and *B. fragilis*, and also for *B. fragilis* and *C. perfringens. S. aureus* and *P. aeruginosa* are among the most common organisms isolated from both acute and chronic wounds, being present in polymicrobial communities of chronic, non-healing wounds (Pastar et al., 2013). Infection with either *S. aureus* or *P. aeruginosa* has been shown to delay wound closure in mouse and rabbit wound healing models (Seth et al., 2012; Zhao et al., 2012; Schierle et al., 2009). The simultaneous infection with *S. aureus* and *P. aeruginosa* significantly impairs wound closure as compared to monospecific biofilms through down-regulation of keratinocyte growth factor 1 (KGF1) expression and increased *S.*

aureus virulence factors *pvl* and *hla* expression in the presence of *P. aeruginosa*, providing *in vivo* evidence that interspecies interactions are operative in promoting bacterial pathogenicity and delayed healing in polymicrobial wound infections. Also, the presence of *P. aeruginosa* in venous leg ulcers can induce ulcer enlargement and/or cause delayed healing (Gjødsbøl et al., 2006).

3. Current therapeutic approaches of wound infections

Wound biofilms are raising difficult clinical and microbiological diagnosis, as well as treatment problems, requiring new and rapid detection methods, as well as combined approaches, such as mechanical debridement and administration of systemic or topical antimicrobial agents and antibiotics or other alternative preventive or therapeutic strategies (Black and Costerton, 2010).

A novel wound diagnosis technology called "bar coding" has been proposed (Tomic-Canic et al., 2008). This method uses samples of chronic wounds fluids and/or tissue biopsies for the identification of chronicisation markers, such as growth factors and their receptors, MMPs, members of the A-catenin/c-myc pathway, and keratinocyte differentiation markers (Tomic-Canic et al., 2008). Growth factors are critically important for coordinating cell–cell and cell–matrix interactions during normal injury repair. Insufficient bioavailability of growth factors, because of diminished synthesis and/or excessive degradation, is a hallmark of chronic wounds. Therefore, exogenous growth factors delivered to non-healing wounds may facilitate cellular responses and lead to timely wound closure (Tatiana et al., 2012).

Wound bar coding can be used for both guiding wound debridement and individual treatment regimens and adjustment, in order to turn the wound microenvironment to a healing phenotype (Tomic-Canic et al., 2008).

Among wound cleansing, debridement has been shown to favor the wound healing by reducing the wound associated biofilm (Nusbaum et al., 2012). The wound debridement could be performed by surgical, biological or enzymatic ways. The biological way is using larvae of the green blowfly species (maggot therapy) (Wolcott et al., 2009), while the enzymatic debridement is using naturally occurring matrix-degrading enzymes, such as papain and collagenase (Ayello and Cuddigan, 2004; Attinger et al., 2006; Turkmen et al., 2009).

Maggot debridement therapy using fly larvae (*e.g. Lucilla sericata*) has revived in the treatment of diabetic foot wound infections, acting by many ways, *i.e.* as physical cleaning agents during the feeding process; (i) by the release of antimicrobial and lytic enzymes with microbicidal activity and also implicated in the degradation of devitalized host tissue which is a potential substratum for microbial adherence, thus significantly reducing the complexity of coaggregating disease-contributing microbial communities; and (ii) by the upregulation of fibroblast growth (Cazander et al., 2010; Margolin and Gialanella, 2010; Prete, 1997).

Along with conventional surgical treatments, the pressure reduction by vacuum assisted therapy favored the clearance of polymicrobial wound infections improving the healing time (O'Connor et al., 2005; Armstrong et al., 2007).

The use of hyperbaric oxygen therapy involving the intermittent inhalation of pure oxygen (100%) at a pressure greater than 1 atm (Lee et al., 1997; Barnes, 2006) could enhance the cellular responses to tissue injury (*e.g.* leukocyte function, fibroblast proliferation, granulation, angiogenesis, and collagen deposition) (Williams, 1997; Zamboni et al., 1997), being recommended in acute traumatic ischemias (*e.g.* crush injuries), clostridial myonecrosis, necrotizing soft tissue infections, and selected nonhealing problem wounds (Ciaravino et al., 1996). At the same time, this therapy increases the respiration of aerobic microorganisms simultaneously inhibiting

the anaerobic bacteria (Williams, 1997). Moreover, the increased respiration rate leads to higher susceptibility to broad-spectrum antimicrobial agents, enhancing the microbicidal effect on the previously dormant biofilm cells (Rani, 2007).

Other alternative antimicrobial therapies, surpassing the antimicrobial resistance phenomenon, are represented by the use of antimicrobial peptides (defensins, magainins, cecropins) (Greener, 1998) and of essential oils which possess antimicrobial properties, honey, sugar paste. The observed benefits of honey in infected wounds may be attributed to the high glucose content and low pH, stimulating the macrophages, the slow and sustained production of hydrogen peroxide by some types of honey, the rich content in flavonoids and aromatic acids with antimicrobial properties, deodorizing effect, due to the production of lactic acid released from the glucose metabolism, in spite of proteinaceous necrotic tissue, resulting in the production of malodorous compounds (Cooper and Molan, 1999; Molan, 1999).

Although the Koch's postulates influenced the global understanding of the infectious processes and the development of specific antimicrobial therapies, they were formulated with reference to infections with a monomicrobial etiology. However, polymicrobial infections represent functional ecosystems that need to be deeply investigated and understood before developing efficient therapeutic strategies (Nelson et al., 2012).

In case of polymicrobial infections several attempts have been made for implementing polymicrobial vaccines, but the results were not so efficient as expected, due to many reasons, such as: (i) biofilm embedded cells have a significantly different and insufficiently elucidated gene expression profile, as compared to their planktonic counterparts, making difficult the selection of vaccine epitopes in order to make sure that immunogenic proteins are expressed during polymicrobial infections (Brady et al., 2006; Rollenhagen et al., 2004; Waite, 2006); (ii) the interspecific interactions are modulating the expression of virulence factors in a certain pathogenic species (Sibley, 2008), as well as of the host antimicrobial and innate immunity genes; (iii) the selection of microbial species targeted by a polymicrobial vaccine is difficult, because two or more species may act synergistically or antagonistically to mediate disease, despite their intrinsic virulent or bending status (Diebel et al., 1999); (iv) eradication of one species from the polymicrobial community may be insufficient to reduce overall disease, as another organism present may fill the niche left behind. Such polymicrobial vaccine strategies should be optimized by directing antigen selection toward shared microbial determinants to simultaneously attenuate the potential virulence of different co-infecting species, in order to diminish the risk of secondary infections.

Besides vaccination, the synbiotics (defined as the combination of a probiotic and a prebiotic) have been used to prevent polymicrobial infections associated to *C. albicans* and *Candida famata* biofilm adherence on PEG devices.

Phage therapy using cocktails of viruses with a specific tropism for bacterial cells may be efficient against polymicrobial biofilm-mediated infections, acting by their bactericidal effects, as well as by encoding lytic enzymes, degrading the bacterial polymeric components (Hughes et al., 1998; Donlan, 2009). Due to their high host specificity, bacteriophages can be used for targeting certain pathogenic or coaggregative species grown in a polymicrobial biofilm growth.

Novel techniques, including photodynamic therapy could successfully eliminate planktonic, biofilm-associated, and multidrug-resistant bacteria (Di Poto et al., 2009).

One of the therapeutic approaches in wound infections is represented by the use of dry or occlusive dressings. The last ones, such as polyurethane films, polyurethane foams, and hydrocolloids, acting to maintain a moist and optimal environment for wound healing,

have been shown to reduce the infection rate (Handfield-Jones et al., 1988; Hutchinson and Lawrence, 1991).

Currently, both the acute chronic wounds of different etiologies are treated using a multistep approach, known as TIME (Schultz et al., 2003): (i) the nonviable tissues (T) from within and around the wound are removed using surgical/chemical/biological debridement; (ii) infection and inflammation (I) are minimized by administering antibiotics and anti-inflammatory drugs; (iii) moisture (M) imbalance is corrected, generally with carefully selected dressings; (iv) epithelialization (E) and granulation tissue formation are promoted by the application of specific therapies, such as growth factors.

The main purpose of a drug delivery system for wound healing would be to protect the active drug from degradation in the wound environment, extend its presence/activity at the site of injury, minimize its systemic absorption, and, if possible, prevent immune responses (Tessmar and Gopferich, 2007).

One of the most investigated protein-based delivery system is represented by collagen that can be easily obtained in large amounts from animal and human sources, has relatively low antigenicity, and can support the growth of a variety of cell types, such as fibroblasts, keratinocytes, and endothelial cells (Cen et al., 2008). Concerning the wound dressings, collagen is used as sponges, skin substitutes containing dermal and/or epidermal cells and collagen-based matrices with synthetic backings. Collagens can be also combined with oxidized regenerated cellulose. Collagen-based dressings are particularly suitable for treatment of chronic wounds, as they have been shown to effectively control wound exudate, inactivate proteases, and can protect exogenously added growth factors from degradation (Cullen et al., 2002; Kakagia et al., 2007; Grumezescu et al., 2013a; Voicu et al., 2013). Collagen-based materials can be used for growth factor delivery (Stompro et al., 1989), such as the recombinant human PDGF-BB becaplermin, which is supplied in a gel form with sodium carboxymethylcellulose serving as a vehicle, being effective in the treatment of both chronic and acute wounds (Cohen and Eaglstein, 2001).

Fibrin is a fibrous protein that forms complex 3-dimensional, biocompatible and biodegradable gel networks stabilized by plasma transglutaminase. It is a major component of the natural wound provisional matrix formed immediately after injury as part of the blood-clotting cascade and plays a key role in initial events of healing. Fibrin gels, structurally similar to native fibrin, can be formed *in vitro* from purified blood plasma containing fibrinogen and thrombin in the presence of calcium chloride and currently FDA approved for use as hemostatic agents, as sealants for colostomy closures, and for skin graft attachment (Janmey et al., 2009; Spotnitz, 2010). The fibrin matrices configuration can be modified by varying the polymerization conditions (pH, salt, and fibrinogen concentration, *etc.*). Several groups have used fibrin gels or scaffolds to deliver growth factors, either alone or in combination with cells into the cutaneous wound bed.

Among the polysaccharide-based matrices for growth factor delivery, carboxymethyl cellulose (CMC) is soluble in water and is used for a large variety of pharmaceutical applications, such as wound-healing gels and fibers, to deliver large and small proteins, such as FGF-2, TGF-β family members (*e.g.* BMP-2 used for bone repair) and as excipient and carrier in the PDGF-BB-containing ointment becaplermin (Regranex), which is approved for usage in diabetic wounds (Leahy and Lawrence, 2007; Ladin, 2000; Kuhn, 2001; Blokhuis, 2009; Grumezescu et al., 2012a).

Chitosan, a polysaccharide consisting of N-acetyl-D-glucosamine and β-(1,4)-linked D-glucosamine produced by the deacetylation of chitin can be chemically modified and used in the form of films, hydrogels, fibers, and dressings, exhibits superior hemostatic ability and antimicrobial and wound-healing properties, and is FDA approved (Kumar et al., 2004; Ueno et al.,

2001; Dai et al., 2009; Chifiriuc et al., 2012; Grumezescu et al., 2011, 2013b; Lin et al., 2013; Huang et al., 2013a; Balaure et al., 2013). Chitosan films and microspheres have been used to deliver a number of growth factors (*e.g.* FGF-2 and EGF) to wounds of different origin, but without significant results, excepting the delivery of FGF-2 incorporated into photo-crosslinkable chitosan dressings, which was able to stimulate wound healing in diabetic mice (Dai et al., 2011; Park et al., 2009; Obara et al., 2003).

Alginate is a linear polysaccharide derived from brown marine algae that can form homopolymers or hetero-polymers and in the presence of Ca^{2+} ion alginates form gels that have been used as excipients in the pharmaceutical industry and later for management of acute and chronic wounds (Lee et al., 2009; Huang et al., 2013b; Grumezescu et al., 2013c). Gel biocompatibility and relatively mild gelation conditions make alginate gels promising vehicles for growth factor and cell delivery. Alginate matrices covalently cross-linked using heparins bound *via* ethylenediamine chemistry allowed a sustained FGF-2 release from the matrix, similar techniques being applied to obtain laminin and elastin-derived peptides for proangiogenic factors delivery in dermal ulcer in a rabbit (Tanihara et al., 2001; Hashimoto et al., 2004).

The synthetic polymers that can be used to deliver growth factors into the wound bed are: poly(α-hydroxyacids–poly(lactic acid), poly(glycolic acid), and poly(lactide-co-glycolide). Poly(lactic acids) (PLA) and poly(glycolic acids) (PGA) are biocompatible and biodegradable polymers that have been FDA approved for a number of biomedical applications and have been proposed for use in drug delivery (Wnek and Bowlin, 2008; Sokolsky-Papkov et al., 2007; Grumezescu et al., 2013d). Poly(lactide-co-glycolic acid) is a component of commercially available skin substitutes (Simamora and Chern, 2006). Poly(α-hydroxyacids) (PHA) have also been tested as carriers for a number of growth factors, as well as their combinations, such as proangiogenic VEGF and PDGF-BB delivered to ischemic hind limbs in nonobese diabetic mice (Sokolsky-Papkov et al., 2007). Polyethylene glycol (PEG) exhibits a high water solubility, biocompatibility, and versatility making them attractive materials for delivery of biologically active molecules, including growth factors, such as VEGF or VEGF and TGF-β1 combinations (Lin and Anseth, 2009).

The problem of protein instability could be eliminated if the resident cells could be stimulated to produce the proteins *in situ*, by gene therapy. The chronic wounds are attractive targets for gene therapy, achieved by nonviral (plasmid DNA), virus-mediated delivery or the direct injection of DNA into the wound bed-associated cells using microneedles or electroporation. The cDNA-coated gold particles could be introduced into the skin or other tissues with a "particle-bombardment device" or "gene gun". The liposomal formulations can also be used to transport negatively charged DNA into living cells (Branski et al., 2009).

Biofilms are implicated in suture-related infections (Leaper et al., 2010; Anghel et al., 2013; Holban et al., 2013; Chifiriuc et al., 2013; Cotar et al., 2013; Grumezescu et al., 2012b; Saviuc et al., 2011), such as post-traumatic endophthalmitis produced by slime-producing *S. epidermidis* (Nucci et al., 2005). The infected surgical wounds lead to increased patient trauma, hospitalization length and treatment costs, estimated in different studies to reach $3937 per infected patient (Johnson and Yu, 1991; Zoutman et al., 1998).

Preventive strategies of biofilms implicated in suture-related infection include prophylactic antibiotics preventing the biofilm initiation, or 'intelligent' coated surfaces (using the antiseptics chlorhexidine, poly-hexamethylene biguanide, octenidine and triclosan) that prevent colonization and/or exhibit antimicrobial properties. Antimicrobial-impregnated implants, which prevent bacterial adhesion and biofilm formation, can avoid long-term, ineffective, systemic antibiotics, reduce the risk of microbial resistance generation and need for implant removal. Sutures made

of silk and Dacron containing cephalosporins and neomycin reduced the number of *S. aureus*, *E. coli*, *Proteus mirabilis*, or *P. aeruginosa* bacterial cells (Rodeheaver et al., 1983). Triclosan-coated poliglecaprone, triclosan-coated polyglactin and PDS with triclosan impregnation reduced the bacterial colonization by *E. coli*, *S. aureus*, S. *epidermidis, K. pneumoniae* in *in vitro* and *in vivo* models (Ming et al., 2007; Storch et al., 2004; USPTO).

4. Conclusions and perspectives

Polymicrobial biofilms represent an understudied and clinically relevant health problem, with the potential to serve as an infectious reservoir for a variety of microorganisms, including bacteria and fungi. The current and future approaches of polymicrobial wound infections management should be based on a better understanding of the complex interactions occurred among different microbial species and between microbes and the infected host, which influence the composition of the biofilm, as well as the clinical outcome of the infection and should consider the following aspects: (i) wound infection is not due to a certain pathogenic species, but to complex interactions between microbial community members; (ii) the *in vivo* expression of virulence factors is different from the intrinsic virulence and antibiotic susceptibility profiles of a single microbial species in planktonic growth state; (iii) the microbial debridement strategy should take into account the deleterious microbiological and immunological consequences of the normal microbiota suppression; (iv) the efficient approach of polymicrobial wound infections should be based on the use of associated, multitarget therapies, with antimicrobial, immunomodulatory and regenerative effects.

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